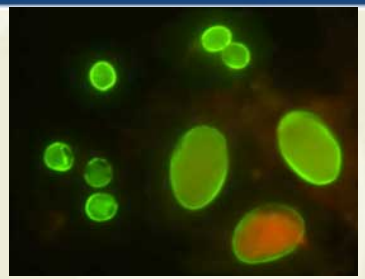

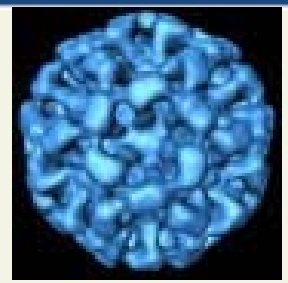

Microbes of Potential Concern in Distribution System Biofilms

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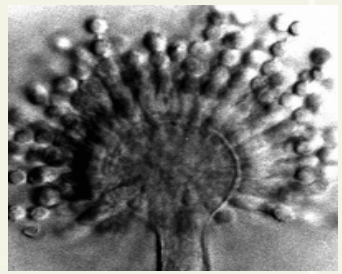


Microorganisms of public health concern in Biofilms

- It is not just bacteria
  - Viruses, protozoa and fungi can contribute to morbidity and mortality
- Immune status is important
  - Immunocompromised individuals have increased susceptibility to infection
  - An opportunistic pathogen is one that usually causes disease only in those whose immune system is compromised

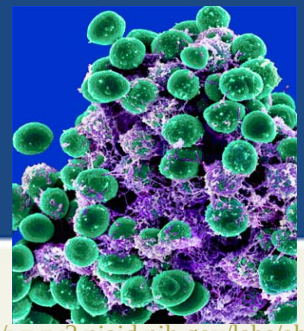
Immune status

- A weakened immune system may be due to:
  - Very young or old age
    - By 2030, nearly 20% of the total U.S. population will be over 65 years of age
  - Cancer therapy
    - the number of persons living with cancer has tripled
      - 3.0 million in 1971
      - 9.8 million in 2001
    - Many are on immunosuppressive medications
  - Pregnancy – 6 million
  - Chronic illness
    - Diabetes – 16 million (~6 % of US population)
    - Cardiovascular disease – 60 million (~22% of US population)
  - Human immunodeficiency virus
    - Over 400,000 individuals in the US are living with AIDS

Bacteria of Health Concern

- Opportunistic bacterial pathogens
  - *Legionella spp.*
  - *Mycobacteria spp.*
  - *Pseudomonas spp*.
  - *Aeromonas spp.*
- Primary bacterial pathogens
  - *Campylobacter spp*.
  - Enterohemorrhagic *E. coli*
  - *Salmonella typhimurium*
  - *Shigella*
  - *Yersinia*
  - *Helicobacter pylori*

Bacteria in Biofilms



http://www3.niaid.nih.gov/labs/aboutlabs/lhbp/pathogenMolecularGeneticsSection/

- Biofilms are remarkably difficult to treat with antimicrobials.
  - Antimicrobials may be readily inactivated or fail to penetrate into the biofilm
  - bacteria within biofilms have increased (up to 1000-fold higher) resistance to antimicrobial compounds
    - even though these same bacteria are sensitive to these agents if grown under planktonic conditions
- Biofilms increase the opportunity for gene transfer between/among bacteria
- Certain species of bacteria communicate with each other within the biofilm.
  - As their density increases, the organisms secrete low molecular weight molecules that signal when the population has reached a critical threshold. This process, called quorum sensing, is responsible for the expression of virulence factors
- Bacteria express new, and sometimes more virulent phenotypes when growing within a biofilm.
- Bacteria embedded within biofilms are resistant to both immunological and non-specific defense mechanisms of the body

Legionella – An important biofilm bacteria

- At least 46 species and 70 serogroups have been identified.
  - *Legionella pneumophila*, an ubiquitous aquatic Gram-negative bacteria that thrives in warm environments (32°- 45°C)
    - causes over 90% of legionnaires' disease (LD) in the United States.
- LD is the more severe form of legionellosis
  - characterized by pneumonia commencing 2-10 days after exposure.
- Pontiac fever is an acute-onset, flu-like, non-pneumonic illness, occurring within a few hours to two days of exposure.

http://www.nrc-cnrc.gc.ca/education/bio/gallery_legionella_e.html

Legionella Transmission

- Person-to-person transmission has not been reported to occur
- Inhalation of contaminated aerosols
  - cooling towers,
  - showers and faucets
  - aspiration of contaminated water
- Legionnaire's disease is an acute respiratory infection which can cause a broad spectrum of symptoms from mild cough and fever to a serious pneumonia

Legionella Incidence

- An estimated 8,000-18,000 cases of *Legionella* occur each year in the United States, but because of under-diagnosis and underreporting, only 2 to10% of estimated cases are identified.
  - Most legionnaires' disease cases are sporadic;
  - 20-25% are nosocomial
  - 10%-20% can be linked to outbreaks.
  - Pontiac fever has been recognized only during outbreaks.
- Death occurs in 10%-15% of legionnaires' disease cases:
  - a substantially higher proportion of fatal cases occur during nosocomial outbreaks.
  - Pontiac fever is a self-limited disease that requires no treatment.

Free-living Protozoa

- Amoebae and Acanthamoebae
  - Intracellular replication of *L. pneumophila* within protozoa play a major role in the transmission of Legionnaires' disease.
    - Protozoa provide habitats for *Legionella* environmental survival and replication
  - Other opportunistic pathogens can also be associated with free-living protozoa
    - *Mycobacterium spp., Pseudomonas spp., Burkhoderia spp., Aeromonas spp., H. pylori* etc

Mycobacterium avium Complex (MAC)

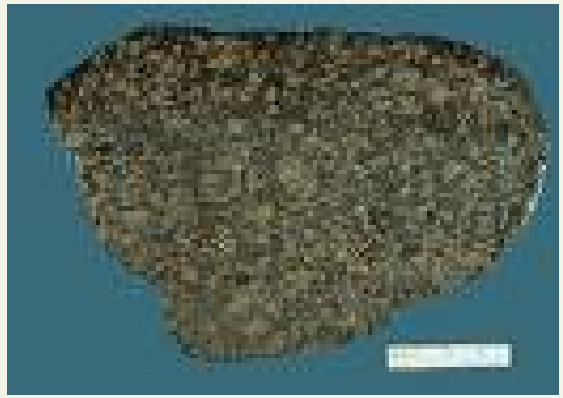
- MAC consists of 28 serovars of two distinct species:
  - *Mycobacterium avium*
  - *Mycobacterium intracellulare*
- Exposure to MAC organisms may occur through
  - contact with or ingestion, aspiration, or aerosolization of potable water containing the organisms.
  - inhalation of air with contaminated soil particles
  - consumption of contaminated food

Mycobacterium avium Complex

- The clinical presentation of MAC infections can include a productive cough, fatigue, fever, weight loss, and night sweats
- The symptoms encountered with MAC infections result from colonization of either the respiratory or the gastrointestinal tract, with possible dissemination to other locations in the body.

Mycobacterium avium Complex

- Disseminated infections are usually associated with HIV infection.
  - Incidence is decreasing among HIV- infected patients as a result of new treatment modalities
    - combination therapy with nucleoside reverse transcriptase inhibitors and protease inhibitors
    - antimycobacterial prophylaxis



http://www.md.huji.ac.il/mirror/webpath/AIDS039.jpg

MAC involving spleen

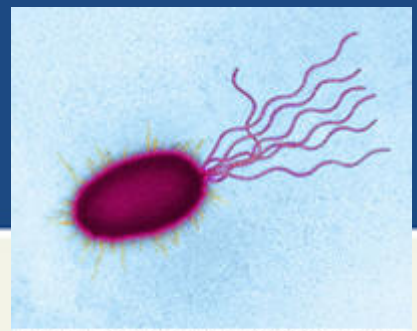
Mycobacterium avium Complex

- *M. avium* can infect individuals with chronic lung conditions
  - bronchiectasis, emphysema and cystic fibrosis
- Lung disease secondary to aerosolized MAC from hot tubs has recently been described in young, immunocompetent individuals.

Mycobacterium avium Complex

- Unlike gastrointestinal pathogens, where fecal indicators such as *E. coli* can be used to indicate their potential presence, no suitable indicators have been identified to signal increasing concentrations of MAC organisms in water systems.

Pseudomonas spp



http://www.ehagroup.com/epidemiology/illnesses/images/pseudomonas-aeruginosa.jpg

- *Pseudomonas aeruginosa* is common in soil, water, and vegetation.
- It is found on the skin of healthy individuals and has been isolated from the throat (5%) and stool (3%) of nonhospitalized patients.
- The gastrointestinal carriage rates increase in hospitalized patients to 20% within 72 hours of admission.

Pseudomonas spp.

- *Pseudomonas aeruginosa* is an opportunistic pathogen
  - respiratory system infections
  - urinary tract infections,
  - Dermatitis & soft tissue infections
  - bacteremia
  - bone and joint infections
  - gastrointestinal infections

http://www.microbes-edu.org/etudiant/imgbgn/pyomain.jpg

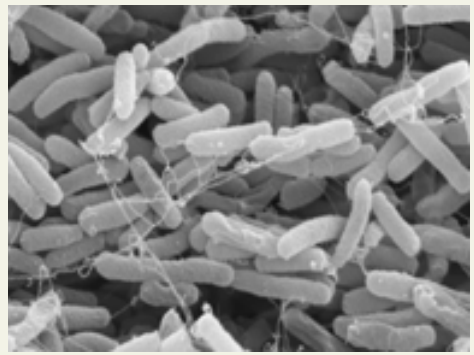
- *Pseudomonas aeruginosa* infection is a serious problem in patients hospitalized with cancer, cystic fibrosis, and burns. The case fatality rate in these patients is 50%
- Many *Pseudomona spp*. are resistant to multiple antibiotics

Aeromonas spp.

- The species principally associated with gastroenteritis are
  - *A. caviae*, *A.hydrophila*, and *A. veronii*
- Aeromonads have been commonly isolated from patients with gastroenteritis although their role in disease causation remains unclear.
  - They are also associated with
    - sepsis and wounds
    - eye, respiratory tract, and other systemic infections
- The pathogenesis of *Aeromonas* infections remains poorly understood
  - They express a range of virulence factors, including attachment mechanisms and produce of a number of toxins.

Primary Bacterial Pathogens

- *Many enteric bacteria have the potential to attach to biofilms where they can aggregate and be protected from disinfection.*
  - *Campylobacter spp*.
  - Enterohemorrhagic *E. coli*
  - *Salmonella typhimurium*
  - *Shigella*
  - *Yersinia*
  - *Helicobacter pylori*



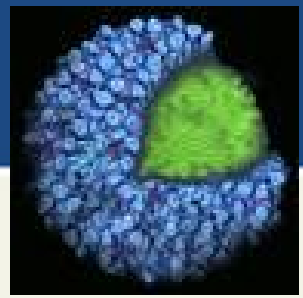
Enteric Viruses

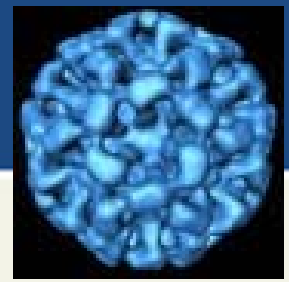
- Transmitted via fecal-oral route – predominantly from human waste
- Viruses cannot replicate outside of specific hosts thus they can accumulate but not grow in a biofilm
  - Protection from disinfectants
  - Aggregation of virus
- Enteric viruses are non-enveloped and thus resistant to environmental degradation and chemical inactivation
  - Persist for weeks to months in cool aqueous environments
- The infectious dose for most human enteric viruses is very low (10-100 particles) and thus even very low levels are of public health concern
- There is minimal to no correlation between bacterial indicators and viruses in potable water

Enteric Viruses of Public Health Concern

- Calicivirus
  - Norovirus and Sapovirus
- Hepatitis A virus
- Adenovirus
- Rotavirus
- Coxsackievirus
- Echovirus
- Astrovirus

Norovirus (NoV)

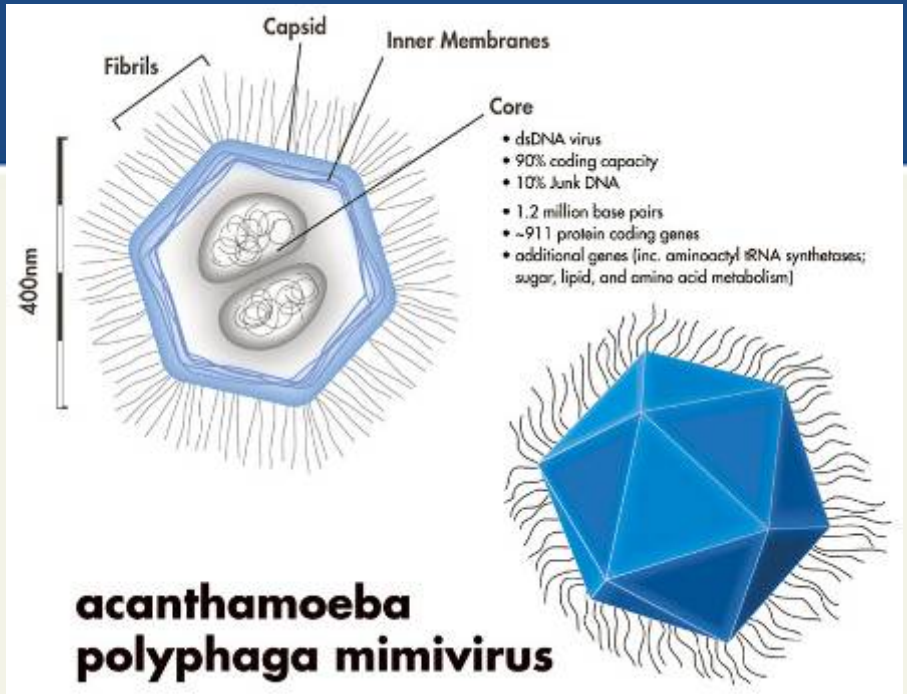




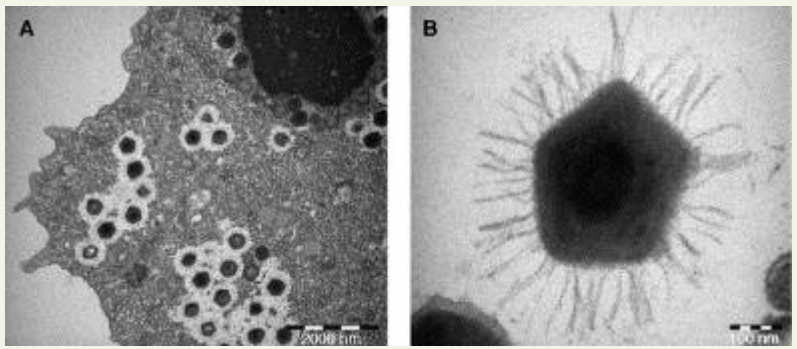
- A genus in *Caliciviridae* family
  - Genetically diverse >150 strains
- The single most important cause of nonbacterial gastroenteritis in US and throughout the world
  - 23 million cases per year in US
- Small round structure, 27-30 *nm* dia.
- Non-enveloped protein capsid, positive sense single strand RNA genome
- Causes self-limiting intestinal illness
  - Major concern in nosocomial settings

Mimivirus

- **Mimivirus (*Acanthamoeba polyphaga*)**
  - **Mimivirus may be a causative agent of some forms of pneumonia**



http://en.wikipedia.org/wiki/Image:Mimivirus.jpg



http://www.stanford.edu/group/virus/mimi/2005/mimipic1.jpg

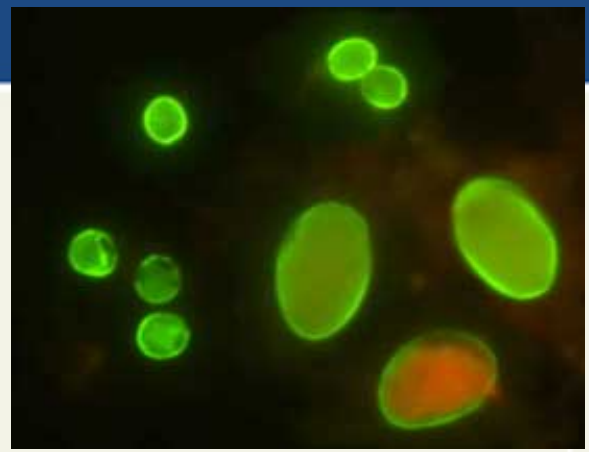
Primary Protozoa

- Like viruses most primary protozoal pathogens cannot replicate outside of specific hosts thus they can accumulate but not grow in a biofilm
  - Present in water in a non-reproductive protective stage (e.g. cyst, oocyst)
- Human pathogenic protozoa are resistant to environmental degradation and chemical inactivation
  - Persist for months in cool aqueous environments
- The infectious dose for most human enteric protozoa is very low (10s-100s of (oo)cysts) and thus even very low levels are of public health concern
- There is minimal to no correlation between bacterial indicators and protozoa in potable water

Protozoa of Public Health Concern

- *Cryptosporidium*
- *Giardia*
- *Microsporidia*
- *Toxoplasma gondii*
- *Cyclospora*
- *Entamoeba*
- *Acanthamoeba*
- *Naegleria*

Cryptosporidium



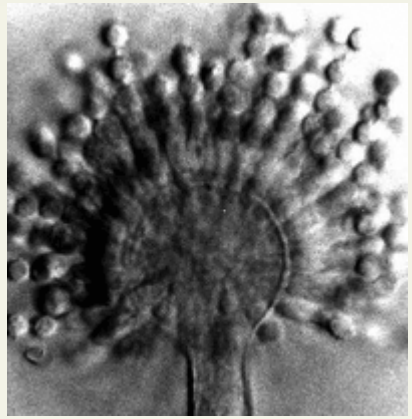
- Oocysts are very infectious
  - Infectious dose 10s to 100s
- 3-11 day incubation (average is 7 days)
- May result in asymptomatic carriage
- Self-limiting in healthy individuals
  - Watery diarrhea without blood
  - Illness lasts 10-14 days
- Immunocompromised patients
  - >50 stools/day with tremendous fluid loss
  - Can be severe and last for months
  - 10-15% of AIDS patients die of complications related to cryptosporidiosis

Fungi

- Fungi are classified as
  - Molds – branching, threadlike filaments
  - Yeasts – single-celled organisms that reproduce by budding
- Fungi are ubiquitous in the environment and have been found in water distribution systems
  - Opportunistic and infrequently cause illness
  - Several spp. can produce toxins

Fungi

- *Aspergillus* spp.
  - *A. fumigatus; A. flavus; A. niger*
    - Pulmonary disease
- *Cryptococcus neoformans*
  - Lung infections, meningitis
- *Candida albicans*
  - Vaginal, urinary and esophageal infections
- *Stachybotrys chartarum*
  - Pulmonary disease



http://www.pall.com/images/flavcf.gif

Detection Strategies

- Many bacteria can enter a viable but not cultivable (VBNC) state making culture analysis challenging
- Culture analysis reflective of infectivity
  - Risk to public health
- Molecular techniques are sensitive and specific but most do not provide information on the infectious nature of the detected microorganism

Control of Microorganisms of Public Health Concern in Biofilms

- The number of immunosuppressed individuals is increasing
  - Increased health risks due to opportunistic pathogens
- Microorganisms other than bacteria are important public health risks
  - Viruses, protozoa, fungi
- Control of microbial contamination requires maintenance of a constant disinfectant residual throughout the water system.
- Treatment strategies targeting free-living amoebae should lead to improved control of *L. pneumophila* and other pathogens.
- Biofilms are unique environments that will continue to be a challenge for the water industry